

**CHAPTER 5. MECHANISMS BY
WHICH CIGARETTE
SMOKE ALTERS THE
STRUCTURE AND
FUNCTION OF THE
LUNG**

CONTENTS

EFFECT OF CIGARETTE SMOKING ON INFLAMMATORY AND IMMUNE PROCESSES IN THE LUNG

Introduction

Effect of Smoking on Numbers and Types of
Inflammatory Cells

Effect of Smoking on the Morphology and Function of
Inflammatory Cells

Emphysema

Populations Deficient in Alpha₁-antitrypsin

Alpha₁-antitrypsin

Proteolytic Enzymes Inducing Emphysematous
Change

Papain

Pancreatic Elastase

Polymorphonuclear Leukocyte Elastase

Alveolar Macrophage Elastase

Protease-Antiprotease Hypothesis

Increased Elastase Owing to the Cellular
Response to Smoke

Number of Cells

Elastase Content

Release

Proximity

Milieu

Decreased Antiprotease Owing to Oxidation

Explanation for Upper Lobe Distribution

Animal Models of Emphysema

Spontaneous Emphysema

Experimentally Induced Emphysema

Oxides of Nitrogen

Cadmium Salts

Cigarette Smoke

The Effects of Smoking on Cellular and Immune Defense
Mechanisms

In Vitro Effects of Cigarette Smoke on Inflammatory
and Immune Effector Cells

The Effect of Cigarette Smoke on Antibody
Production

EFFECTS OF CIGARETTE SMOKE ON AIRWAY MUCOCILIARY FUNCTION

Introduction

Normal Mucociliary Function

Cilia

Mucus

Mucociliary Interaction

Effects of Cigarette Smoke on Mucociliary Function

Short-Term Exposure

Cilia

Mucus

Mucociliary Interaction

Long-Term Exposure

Cilia

Mucus

Mucociliary Interaction

Fractionation and Filtering of Cigarette Smoke

Effects of Filters

Mucociliary Function in Chronic Bronchitis

Cilia

Mucus

Mucociliary Interaction

Summary and Conclusions

References

EFFECT OF CIGARETTE SMOKING ON INFLAMMATORY AND IMMUNE PROCESSES IN THE LUNG

Cigarette smoke is a complex mixture of several thousand different constituents that may produce physiologic and pathologic changes. This discussion focuses on the cellular and immune responses of the lung to cigarette smoke, the mechanism by which smoking can cause emphysema, and the impact of smoking on mucociliary clearance. The last 20 years have witnessed dramatically increased understanding of cigarette-induced lung injury, particularly emphysema, thus enhancing our understanding of the process by which cigarette smoking can lead to emphysema.

Introduction

Inhalation of cigarette smoke markedly alters the inflammatory and immune processes in the lung, leading to increases in the total number of inflammatory cells and to changes in cell type and function. These effects of cigarette smoke on lung inflammatory cells may play a role in decreased pulmonary host defenses against various microorganisms and the development of lung cancer, chronic bronchitis, and pulmonary emphysema (USPHS 1971, 1972, 1973, 1974, 1975; USDHHS 1981).

Effect of Smoking on Numbers and Types of Inflammatory Cells

One of the most consistently observed effects of cigarette smoking on the lung is a marked increase in the numbers of inflammatory cells, especially at sites of disease. Increased numbers of inflammatory cells have been seen in pathological studies of the lungs of cigarette smokers, as well as in lungs of animals exposed to cigarette smoke. In addition, increased numbers of inflammatory cells occur in bronchoalveolar lavage fluid of cigarette smokers and in lavage fluid of animals exposed to cigarette smoke.

Spain and Kaufman (1953) noted inflammatory changes in the lung bronchi of cigarette smokers. Later, Anderson and Foraker (1961) described the presence of an alveolitis, and McLean (1959) described the presence of a bronchiolitis in these patients. In an autopsy study of patients with early emphysema (McLaughlin and Tueller 1971), numerous abnormal, brownish-pigmented alveolar macrophages were found in adjacent, otherwise intact parenchyma, but none were found in normal lungs. Identical pigmented macrophages were found in the sputum of patients obtained from apparently healthy cigarette smokers. The frequency of occurrence of these macrophages in the tissue appeared to be related to the

number of cigarettes consumed. Niewoehner et al. (1974) evaluated the lungs of young smokers and controls of comparable age from a population that had experienced sudden nonhospital deaths. In smokers, a characteristic lesion occurred in the form of respiratory bronchiolitis associated with clusters of pigmented alveolar macrophages. This lesion was present in the lungs of all smokers studied, but was rarely seen in nonsmokers. Lungs of smokers also showed small, but significant, increases in mural inflammatory cells and denuded epithelium in the membranous bronchioles as compared with controls. The researchers suggested that this respiratory bronchiolitis may be a precursor of emphysema and may be responsible for the subtle functional abnormalities that are observed in young smokers. Mitchell et al. (1976) also noted the presence of significant amounts of inflammation in the small airways of cigarette smoker lungs, and Cosio et al. (1978) suggested that the primary lesion in the small airways was a progressive inflammatory reaction, leading to fibrosis with connective tissue deposition in the airway walls. These lesions were closely correlated with abnormalities in pulmonary function.

As noted above, most early investigators concentrated on the role of the increased numbers of pigmented alveolar macrophages present at disease sites in cigarette smokers. These pigmented macrophages, because of their numbers and prominent coloration on histologic sections, were initially the sole focus of research on the inflammatory response in these patients. More recently, however, Ludwig et al. (1983) evaluated the relationship between cigarette smoking and the accumulation of neutrophils in the lungs of smoking and nonsmoking humans. Human lungs were obtained from autopsies of 10 cigarette smokers and 5 nonsmokers who experienced nonhospital death. These studies indicated a marked increase in neutrophil infiltration in the lungs of cigarette smokers compared with nonsmokers, and identified the site of the accumulation as the alveolar septa. Neutrophils were found in the alveolar walls of smokers both with and without emphysema. The researchers concluded that a marked neutrophil accumulation occurs in the lungs of cigarette smokers, that it precedes the development of emphysema, and that it continues once emphysema is established. They further suggested that the neutrophils may play a role in the destruction of the alveolar septa of the lungs in cigarette smokers. The presence of increased numbers of neutrophils in cigarette smokers' lungs has also been documented by extracting inflammatory cells from open lung biopsies of smokers and nonsmokers (Hunninghake and Crystal 1983). A higher percentage of these inflammatory cells were neutrophils in smokers compared with nonsmokers. Finally, the association between cigarette smoking and increased numbers of inflammatory cells, including neutrophils, at disease sites has also

been confirmed in numerous animal studies (Frasca et al. 1971; Dahlgren et al. 1972; Rylander 1974; Park et al. 1977).

Increased numbers of inflammatory cells in the lungs of smokers, as compared with nonsmokers, have also been observed by all investigators performing bronchoalveolar lavage studies (Davis et al. 1976; Demarest et al. 1979; Harris et al. 1970, 1975; Hunninghake et al. 1979a, 1980a; Hunninghake and Crystal 1983; Hunninghake and Gadek 1981-1982; Hunninghake and Moseley, in press; Reynolds et al. 1977; Reynolds and Newball 1974, 1976; Rodriguez et al. 1977; Warr et al. 1976, 1977; Warr and Martin 1974, 1978). Such increases have been detected additionally in lavage fluid of animals chronically exposed to cigarette smoke (Davies et al. 1977; Flint et al. 1971; Holt et al. 1973). The majority of these studies have demonstrated increases in both the number of macrophages and the number of neutrophils, although Hoidal and Niewoehner (1982) found increases only in the former.

The presence of neutrophils in the lungs of cigarette smokers is of interest because these cells contain elastase, an enzyme believed to be important in the pathogenesis of emphysema (Lieberman 1976; Karlinsky and Snider 1978; Kuhn and Senior 1978; Carp and Janoff 1978; Snider and Korthy 1978; Schuyler et al. 1978; Janoff et al. 1977; Hunninghake et al. 1979a; Hunninghake and Crystal 1983; Hunninghake and Gadek 1981-1982; Hunninghake and Mosley 1984; Laurell and Eriksson 1963). Alveolar macrophages have also been implicated as a source of an elastase-like metalloprotease (Harris et al. 1975; Rodriguez et al. 1977). This enzyme is not inhibited by α_1 -antitrypsin (α_1 AT) (Banda and Werb 1981), the major anti-elastase in the lower respiratory tract (Gadek et al. 1981). Although macrophages are clearly present in large numbers in the alveolar structures of smokers (Niewoehner et al. 1974; Harris et al. 1975), several lines of evidence suggest that neutrophils may play a significant and perhaps more important role in increasing the elastase burden of the lungs.

First, neutrophils store and release significantly more elastase than do alveolar macrophages (Barrett 1977; Rodriguez et al. 1977; Levine et al. 1976). Comparative estimates of elastase production by human neutrophils and alveolar macrophages suggest that neutrophils are at least 1,000 times more potent elastase producers (Janoff et al. 1979).

Second, although alveolar macrophages of cigarette smokers have been shown to release elastase in vitro (Rodriguez et al. 1977), it is not clear whether the elastase was produced by these cells or was secreted by other types of cells, such as neutrophils, and subsequently ingested by the macrophages (Janoff et al. 1977). In this regard, recent studies by Campbell et al. (1979) and McGowan et al. (1983) have shown that alveolar macrophages are capable of phagocytosing

neutrophil elastase via a receptor-mediated mechanism; some of the elastase remains enzymatically active for up to 48 hours. These findings suggest that alveolar macrophages may, in fact, be capable of both decreasing and increasing the protease burden of the lung.

Third, once a neutrophil has left its vascular space, its lifespan is only a few hours; when the neutrophil dies, it may release at least a portion of its preformed enzymes, including elastase. Thus, when a neutrophil is present within a tissue, it is possible that the tissue will be exposed not only to the elastase secreted by the neutrophil while it is functional, but also to the elastase stored by the neutrophil and released when the neutrophil disintegrates. In this context, the finding that neutrophils represent only a small percentage of all inflammatory and immune effector cells in the smoker's lungs would not preclude the smoker's exposure to a large chronic burden of neutrophil elastase. In contrast, the alveolar macrophage has a half-life of months to years (Thomas et al. 1976), and it stores little, if any, elastase (Rodriguez et al. 1977; Levine et al. 1976).

Macrophages may also play an important role in this process by secreting a potent chemotactic factor for neutrophils (Hunninghake and Crystal 1983). This hypothesis is supported by the following observation: alveolar macrophages of cigarette smokers spontaneously release a chemotactic factor for neutrophils, whereas alveolar macrophages of nonsmokers do not. In addition, *in vitro* exposure to cigarette smoke particulates results in the release of a chemotactic factor from the alveolar macrophages of nonsmokers. The migration of neutrophils to the lung in response to the chemotactic factor may be augmented by factors in cigarette smoke. In this regard, McCusker et al. (1983) have shown that nicotine is a potent chemokinetic factor for neutrophils, enhancing the migration of these cells to other chemotactic factors. Once neutrophils are present in the lung, they may release elastase, because both cigarette smoke (Blue and Janoff 1978) and the macrophage-derived chemotactic factor stimulate these cells to release the enzyme (Gadek et al. 1979a, b).

The postulated release of elastase by neutrophils could also partly explain how the number of macrophages are increased in this disorder. Fragments of elastin (which are probably generated by the release of neutrophil elastase at sites of disease activity) are potent chemoattractants for blood monocytes, the precursors of alveolar macrophages (Senior et al. 1980; Hunninghake et al. 1981). These fragments of elastin possess no chemotactic activity for neutrophils.

Effect of Smoking on the Morphology and Function of Inflammatory Cells

No size differences have been observed between alveolar macrophages from smokers and those from nonsmokers when the cells are

fixed in suspension immediately after bronchoalveolar lavage (Table 1). Harris and coworkers (1970) observed a mean size of 23.3 μm (range, 10 to 47 μm) for nonsmokers and 26.4 μm (range, 12 to 53 μm) for smokers. Reynolds and Newball (1974), using similar methods, did not find any size differences between smoker and nonsmoker alveolar macrophages.

The morphology of smoker macrophages clearly differs, however, from that of nonsmokers (Table 1). Macrophages of smokers show increased numbers of large lysosomes, phagolysosomes, endoplasmic reticulum, ribosomes, and Golgi vesicles (Golde 1977; McLemore et al. 1977; Martin 1973; Warr and Martin 1978; Rasp et al. 1978; Pratt et al. 1971; Brody and Craighead 1975). These findings are generally associated with activated mononuclear phagocytes, and these macrophages have probably become activated by the ingestion of the particulates present in cigarette smoke. Smoker macrophages have pigmented inclusions that appear to have platelike or needlelike configurations when seen by electronmicroscopy (Golde 1977; Warr and Martin 1978; Pratt et al. 1971; Brody and Craighead 1975). Studies of the nature of these inclusions by X-ray analysis suggest they may be, at least in part, particulates of aluminum silicate (Brody and Craighead 1975). Together with *in vitro* studies showing that alveolar macrophages are activated following phagocytosis of particulates (Hunninghake et al. 1980a), these findings are compatible with the notion that macrophages of smokers are activated *in vivo*.

Alveolar macrophages from cigarette smokers have an increased ability to generate superoxide anion (Hoidal et al. 1979a, 1980, 1981), the functional effects of which include an increased capacity to kill lung fibroblasts. These observations suggest that alveolar macrophages from cigarette smokers are increasingly able to injure lung parenchymal cells, and that they may contribute to the observed loss of lung cells in the alveoli of patients with pulmonary emphysema.

A variety of other effector functions of smokers' alveolar macrophages have also been evaluated (Table 1). Alveolar macrophages from cigarette smokers appear to have a normal or increased ability to migrate in response to chemotactic factors (Demarest et al. 1979; Warr and Martin 1974). They differ, however, from normal alveolar macrophages in several other respects: for example, increased glucose utilization has been reported in some studies (Harris et al. 1970), but was normal in others (Hoidal et al. 1979a). Oxygen consumption has been reported to be normal (Hoidal et al. 1979a), but the protein content of these cells has been increased (Harris et al. 1975; Warr and Martin 1978). Alveolar macrophages from smokers release less PGE_2 and thromboxane B_2 than normal macrophages (Laviolette et al. 1981), suggesting that cigarette smoking induces a lesion in phospholipid hydrolysis or the mecha-

TABLE 1.—Cigarette-smoking-induced abnormalities in the inflammatory and immune effector systems > within human alveolar structures

Parameter	Findings in smokers
Cell types present	
Total number of cells	Increased
Percent polymorphonuclear leukocytes	Increased
Percent T lymphocytes	Increased or normal
Percent B lymphocytes	Normal
Lymphocyte function	
Response to mitogens	Decreased
Macrophage structure	
Diameter	Normal
Ruffling of cell surface	Decreased
Number and size of cytoplasmic structures	Increased
Abnormal cytoplasmic inclusions	Pigmented inclusions, particulates with plate or needle-like configuration, presence of aluminum silicate
Macrophage properties and function	
Surface receptors	
IgG-Fc	Normal
C3b	Decreased
Phagocytosis and killing of microorganisms	
Bacteria	Normal or decreased
Fungi	Normal
Effector and accessory cell function	
responsiveness to chemotactic factors	
Casein	Increased
Activated serum	Normal
Function as accessory cell to lymphocytes	Decreased
Responsiveness to MIF	Decreased
Production of neutrophil chemotactic factor	Increased
Secretion of superoxide anion	Increased
Secretion of elastase	Increased
Release of prostaglandin E ₂ and thromboxane B ₂	Decreased
Miscellaneous properties and function	
Glucose utilization	Increased or normal
Oxygen consumption	Normal
Protein content	Increased
Content of various enzymes	
Elastase	Increased
Acid protease	Increased
Neutral protease	Normal
Esterase	Increased
Acid phosphatase	Increased
β-glucuronidase	Increased
Lysozyme	Normal or increased
Aryl hydrocarbon hydroxylase	Increased
Angiotensin-converting enzyme	Increased
Spreading and adherence properties	Increased in presence of serum, decreased nylon adherence
Pinocytosis	Decreased
Content of α ₁ -antiproteinase	Increased

SOURCE: Adapted from Hunninghake et al. (1979).

nism regulating hydrolysis. Smoker macrophages also appear to have increased amounts of various enzymes, including acid protease (Harris et al. 1975), neutral protease (Harris et al. 1975), esterase (Harris et al. 1975), acid phosphatase (Martin 1973), angiotensin-converting enzyme (Hinman et al. 1979), β -glucuronidase (Martin 1973), lysozyme (Martin 1973), and arylhydrocarbon hydrolase (Cantrell et al. 1973; Harris et al. 1978; McLemore et al. 1977b, c, 1978; McLemore and Martin 1977). The functional significance of increased amounts of these enzymes is not entirely clear.

In addition to its effects on the inflammatory and immune effector cells in the lung, cigarette smoke may also affect the composition of epithelial surface fluid. For example, some investigators have found that the amount of immunoglobulin G (IgG) present in lavage fluid is increased (Reynolds and Newball 1974); others have noted normal levels (Warr et al. 1977). Interestingly, cigarette smoking appears to cause a significant decrease in the secretory component of immunoglobulin A (IgA) in the lavage fluid of some people who smoke cigarettes (Merrill et al. 1980). This effect most likely indicates a subtle injury to the epithelium of the lung that produces this factor. The only additional factors that have been reported to be abnormal in lavage fluid of cigarette smokers are an increase in the amounts of fibronectin (Villiger et al. 1981) and a decrease in the function, but not the amount, of α_1 AT (Gadek et al. 1979; Janoff et al. 1979). This latter finding has been disputed by others (Stone et al. 1983).

Emphysema

A number of lines of evidence link the cellular changes described above with the development of emphysema. They include observations in populations deficient in α_1 AT, in animal models of emphysema, and most important, in human cigarette smokers.

Populations Deficient in Alpha₁-antitrypsin

Eriksson (1965) described the characteristic features of α_1 AT-deficiency-associated lung disease. Approximately 60 percent of affected individuals develop symptoms of airways obstruction by age 40, and 90 percent by age 50. Excluding the influence of cigarette smoking, there is no sexual predominance of disease. Kueppers and Black (1974) found that dyspnea occurred a decade earlier in cigarette smokers (35 years in smokers versus 44 years in nonsmokers), and estimated that 70 to 80 percent of all PiZZ persons (where Pi = protease inhibitor) will develop lung disease. Larsson (1978) has projected that nearly 60 percent of PiZZ people will ultimately die of lung-related disease.

Orell and Mazodier (1972) reviewed the morphologic features of α_1 AT-deficiency-associated emphysema and found primarily the

panacinar or panlobular form. Emphysematous lesions may be distributed uniformly throughout the lungs (Orell and Mazodier 1972), but frequently show a predominant lower lobe distribution (Greenberg et al 1973).

In people genetically deficient in α_1 AT, the increased numbers of inflammatory cells found in the lungs of smokers probably present an increased elastase burden to the lung and magnify the protease-antiprotease imbalance. This may explain the deleterious effects of cigarette smoke in this population. Kueppers and Black (1974) reviewed data on the impact of cigarette smoking in people severely deficient in α_1 AT and concluded that, in addition to experiencing earlier onset of respiratory symptoms and pulmonary function abnormalities, cigarette smokers die at an earlier age from respiratory failure than similarly afflicted nonsmokers. The increased prevalence of emphysema in populations deficient in α_1 AT, plus the exacerbation of this lung disease by smoking, suggests that protease-antiprotease imbalance may also play a role in the development of emphysema by smokers who are not deficient in α_1 AT. This suggestion has resulted in a substantial body of research that has characterized α_1 AT, defined the nature of elastase-induced emphysema, and clarified and supported the protease-antiprotease hypothesis of cigarette-induced emphysematous lung injury.

Alpha₁-antitrypsin

The deficient constituent of α_1 -globulin was initially described by Schultze et al. (1955) as α_1 -3,5-glycoprotein but later renamed α_1 -antitrypsin (α_1 AT) when it was found to inhibit trypsin activity (Schultze et al. 1962). Subsequently, α_1 AT has been shown to inhibit a variety of proteolytic enzymes including neutrophil elastase (Ohlsson 1971), neutrophil collagenase (Tokoro et al. 1972; Ohlsson 1971), cathepsin-G (Travis et al. 1978), chymotrypsin (Travis et al. 1978; Rimon et al. 1966), plasmin (Rimon et al. 1966), thrombin (Rimon et al. 1966), Hageman factor cofactor (Crawford and Ogston 1974), coagulation factor XI (Heck and Kaplan 1974), acrosin and kallikrein (Fritz et al. 1972a, b), urokinase (Crawford and Ogston 1974; Clemmensen and Christensen 1976), and renin (Scharpe et al. 1976). Although the range of proteases inhibited by α_1 AT appears broad, the association rate constants of these enzymes for α_1 AT differ (leukocyte elastase > chymotrypsin > cathepsin-G > trypsin > plasmin > thrombin) (Beatty et al. 1980), and the inhibitory role of α_1 AT against enzymes with low association rate constants, such as trypsin, may be negligible. The names α_1 -protease inhibitor or α_1 -proteinase inhibitor better describe this broader range of inhibitory functions and are preferred by some authors. In deference to historical usage and in accord with the recommendations of the

Nomenclature Meeting for this substance (Cox et al. 1983), the name α_1 AT has been retained in this discussion.

The inhibitor α_1 AT is a polymorphic plasma protein (Fagerhol and Cox 1981; Cox and Celhoffer 1974; Cox et al. 1980; Cox 1981; Fagerhol and Braend 1965) encoded by two codominant autosomal alleles and inherited as a single Mendelian trait. The basal serum concentration is genetically determined (Eriksson 1964; Kueppers et al. 1964; Fagerhol and Gedde-Dahl 1969; Talamo et al. 1966). More than 31 allelic variants or Pi types (where Pi, or protease inhibitor, is the symbol assigned the genetic locus of the α_1 AT allele) have been identified (Cox and Celhoffer 1974; Cox et al. 1980; Cox 1981). The variants are designated by capital letters, B through Z, corresponding to their approximate electrophoretic mobility, relative to the anode, in acid starch gel electrophoresis or their relative positions on polyacrylamide isoelectric focusing. New variants are named according to the conventions established by the Fifth International Workshop on Gene Mapping and the Nomenclature Meeting for α_1 AT (Cox et al. 1980).

The M allele (PiM) has a gene frequency of about 0.9 and is the most common Pi type in all populations tested (Kueppers 1978). The α_1 AT serum concentration in PiMM homozygotes is between 1.3 and 2.2 g/liter (depending on the method of measurement and the purity of standard) (Kueppers 1968; Jeppsson et al. 1978a) and, by convention, defines normal. Pi types with decreased circulating levels of α_1 AT include (serum concentration expressed as percent normal) null 0% (Feldman et al. 1975; Talamo et al. 1973), Mmalton and Mduarte 12% (Cox 1976; Lieberman et al. 1976), Z 15% (Laurell and Eriksson 1963; Fagerhol and Laurell 1970), P 30% (Fagerhol and Hauge 1969), S 60% (Fagerhol 1969), and I 68% (Arnaud et al. 1978).

PiZ was the first variant recognized (Laurell and Eriksson 1963) and is the Pi type most frequently associated with a serum deficiency of α_1 AT (Kueppers 1978). Its allele frequency varies markedly between different ethnic and racial groups. In the United States, the allele frequency is greater than 0.010 in whites but nearly zero in blacks (Kueppers 1978). Approximately 1 in 2,000 whites is homozygous for the Z gene (Laurell and Sveger 1975).

Although a decrease in hepatic synthesis is probably the major mechanism for quantitatively significant reductions in serum α_1 AT, the factors that modulate such synthesis are only partially understood (Morse 1978). Impaired hepatic secretion, as evidenced by the presence of intrahepatic cytoplasmic inclusions containing accumulations of α_1 AT polypeptides (Blenkensopp and Haffenden 1977), occurs in persons with the PiZ genotype. It is uncertain if these intrahepatic inclusions exert a negative feedback inhibition on the hepatocyte and thereby retard biosynthesis of α_1 AT. Intrahepatic inclusions are not found with the S and null Pi types (Carrell et al.

1982), suggesting that decreased synthesis, independent of impaired secretion, is primarily responsible for the reduced serum levels of α_1 AT. Catabolic studies of the PiM and PiZ proteins have identified similar half-lives in the circulation, 6 to 7 days and 5 days, respectively (Laurell et al. 1977; Jeppsson et al. 1978b). It is therefore unlikely that accelerated peripheral catabolism contributes significantly to serum deficiencies in α_1 AT.

In addition to quantitative deficiencies in serum α_1 AT, a reduction in serum inhibitory capacity could also result from a loss in the functional activity of α_1 AT. Most genetic variants, however, are functionally equivalent to normal α_1 AT (PiMM) in their capacities to inhibit both trypsin and elastin (Billingsley and Cox 1982).

The inhibitor α_1 AT is a low molecular weight (51,000 daltons) (Mega et al. 1980; Carrell et al. 1981; Chan et al. 1976; Pannell et al. 1974; Jeppsson et al. 1978) protein comprised of a single polypeptide chain containing 394 amino acid residues. Three carbohydrate side chains are attached, each containing terminal sialic acid residues (Mega et al. 1980; Carrell et al. 1981). The α_1 AT reacts stoichiometrically with free protease in a ratio of 1:1; one mole of α_1 AT inhibits one mole of protease and yields a stable complex (Cohen 1973). An in vitro study (James and Cohen 1978) found, however, that complete inhibition of elastase requires molar ratios of α_1 AT to elastase greater than 2.2:1. This phenomenon may be explained by elastase having two major sites of attack on α_1 AT. Attack against one site leads to a conformational change in α_1 AT and inhibition of elastase, whereas attack against the other site results in cleavage and inactivation of α_1 AT. The α_1 AT-protease complexes that form during protease inhibition are not reutilized by the body (Balldin et al. 1978), and the body supplies of α_1 AT are replenished via de novo synthesis by the liver.

In addition to hepatic biosynthesis, α_1 AT is synthesized by at least two other endogenous sources. Both human peripheral lymphocytes and rat alveolar macrophages have been shown to synthesize α_1 AT. Ikuta et al. (1982) demonstrated that concanavalin A-stimulated monocytes interact with human peripheral lymphocytes, causing a threefold increase in α_1 AT synthesis. White et al. (1981) cultured rat alveolar macrophages and recovered newly synthesized radio-labeled (^{35}S) α_1 AT from the cell culture medium. Macrophages and lymphocytes, by virtue of their close physical proximity to the sites of connective tissue injury, may play a significant role in defense against proteolytic destruction. The physiologic significance of extrahepatic synthesis of α_1 AT remains speculative, however.

While certain chemical and physiological aspects of α_1 AT are clear, the exact biochemical mechanism by which it causes protease inhibition is uncertain. It is generally agreed that the reactive center of α_1 AT is located on a single serine-methionine segment peptide.

bond on the carboxyl-terminal end (Carrell et al. 1982; Kurachi et al. 1981).

Proteolytic Enzymes Inducing Emphysematous Change

Proteolytic enzymes have been a major focus of investigation following the demonstration by Gross et al. (1965) of papain's ability to induce emphysematous changes in rats.

Papain

Papain, a proteolytic enzyme with a broad range of substrate specificities (Bergmann and Fruton 1941; Kimmel and Smith 1953), reproducibly causes emphysema-like lesions in a variety of experimental animals following aerosolization or intratracheal instillation (Gross et al. 1965; Palecek et al. 1967; Goldring et al. 1968; Caldwell 1971; Pushpakom et al. 1970; Marco et al. 1969). A number of studies have helped to clarify the critical importance of elastolysis in papain-induced emphysema.

Snider et al. (1974) tested amorphous and crystalline forms of papain and found that the emphysema-inducing properties of these preparations were directly proportional to their abilities to degrade and solubilize elastin. Heat inactivation of papain destroyed its emphysema-inducing capabilities. Similarly, intratracheal pretreatment of hamsters with human α_1 AT, an inhibitor of papain elastolytic activity, ameliorates papain-induced emphysematous changes (Martorana and Share 1976). Furthermore, Blackwood et al. (1973) showed that the elastolytic activities of several microbial enzymes, rather than their nonspecific protease activities, correlate best with the enzyme's ability to induce emphysematous changes following intravenous administration to rats. Snider et al. (1977) showed that enzymes lacking elastolytic activity, such as collagenase or trypsin, do not produce emphysema in hamsters.

Whereas these studies support the notion that the early histologic changes induced by papain are a direct consequence of its elastolytic activity, they do not preclude the possibility that endogenous factors may contribute to subsequent disease progression. Snider and Sherter (1977) noted a gradual increase in static lung volumes in hamsters following a single intratracheal injection of pancreatic elastase. Stone et al. (1979) followed the fate of tritium-labeled pancreatic elastase and found that enzymatically active preparations are retained longer within the lung than inactive preparations, and that 14 C-guanidated elastase remains bound to lung matrix for at least 96 hours. This suggests that tissue-bound elastase may continue to digest elastin for extended periods of time. Martorana et al. (1982) found no progression in the mean linear intercept measurements or internal surface areas in the lungs of papain-treated dogs between 3

and 6 months after treatment. However, the mean pulmonary arterial pressure and pulmonary arteriolar resistance did increase during this interval.

Papain-treated animals exhibit the expected physiologic changes of emphysema: increased RV, FRC, and TLC, decreased elastic recoil, increased static lung compliance at middle and low lung volumes, and reduced diffusing capacity (DL_{COVA} and DL_{CO}) (Caldwell 1971; Pushpakom et al. 1970; Marco et al. 1969; Giles et al. 1970; Johanson and Pierce 1973). Studies by Koblitz et al. (1982) have shown that following papain administration the elastic fibers are disrupted and that the elastin content of the lung initially decreases, but later returns to normal after a period of accelerated synthesis. The newly synthesized fibers are disordered; however (Kuhn and Senior 1978; Kuhn and Starcher 1980).

Pancreatic Elastase

The ability of porcine pancreatic elastase to rapidly hydrolyze insoluble elastin (Partridge and Davis 1955) and its commercial availability in a highly purified crystalline form have led to its extensive use as an experimental agent for inducing emphysema in animals (Karlinsky and Snider 1978). Lesions resembling human panacinar emphysema can be induced in hamsters within 2 hours of intratracheal instillation of pancreatic elastase (Kaplan et al. 1973). The severity of the lesions, as assessed by histologic or physiologic criteria, is dose related (Raub et al. 1982), with adult animals being more susceptible to pancreatic elastase than young animals (Lucey and Clark 1982). Within a few hours of intratracheal instillation in hamsters, hemorrhagic lesions develop and an influx of polymorphonuclear leukocytes is seen (Hayes et al. 1975; Kuhn and Tavassoli 1976). Digestion of elastin fibers is apparent in the pleura and in the alveolar walls by 4 hours, but is more extensive at 24 and 48 hours (Kuhn et al. 1976). By day 4, there is a diminution in the number of polymorphonuclear leukocytes (PMNs), but many macrophages remain (Morris et al. 1981). The hemorrhage and cellular infiltration resolves within 3 weeks, and the ensuing lesions resemble panacinar emphysema (Kuhn et al. 1976). Over 95 percent of the detectable urinary excretion of desmosine and isodesmosine, amino acid markers of in vivo elastolysis, appears within 2 days of elastase instillation; only small amounts can be detected by day 3 (Goldstein and Starcher 1977). Kucich et al. (1980) developed a hemagglutination inhibition assay to measure elastin-derived peptides in serum, and found that elastin-derived peptides could be detected in the serum of dogs for a period of 12 days following administration of a 25 to 50 mg dose of porcine pancreatic elastase and for 40 days following a 100 mg dose. Janoff et al. (1983b) found increases in urinary desmosine excretion during the first 48 hours following endobronchi-

al instillation of pancreatic elastase to sheep; increases in mean linear intercepts and decreases in lung ventilation and perfusion were found after 4 weeks. All changes correlated positively with the elastase dose. Studies have shown a decrease in the lung elastin content within the first 24 hours of intratracheal injection of elastase (Kuhn et al. 1976; Ip et al. 1980; Goldstein and Starcher 1977). Physiologic studies (Snider and Sherter 1977; Snider et al. 1977) of experimental animals after pancreatic elastase administration have shown increases in the lung compliances and in the volume of air within the lungs at specified transpulmonary pressures (25 and -20 cm H²O). These physiologic alterations appear to progress in severity for about 26 weeks following exposure to elastase (Snider and Sherter 1977).

In spite of substantial experimental verification of ability of pancreatic elastase to induce emphysematous changes in animals following intratracheal instillation, there is little evidence implicating endogenous pancreatic elastase in the pathogenesis of pulmonary emphysema in humans. A serine endopeptidase of pancreatic origin (elastase 2) has been shown to circulate in human blood (Geokas et al. 1977). However, the enzyme is rapidly bound to serum inhibitors α_1 AT and α_2 -macroglobulin (α_2 M) and inactivated (Gustavsson et al. 1980). Although α_2 M-elastase complexes retain enzymatic activity against low molecular weight synthetic elastin substrates (N-succinyl-L-alanyl-L-alanyl-L-alanine-4-nitroanilide) (Twumasi and Liener 1977; Barrett and Starkey 1973); high molecular weight proteins such as elastin are prevented from reaching the enzyme and are not hydrolyzed (Barrett and Starkey 1973).

Attempts to induce emphysematous changes via the intravenous injection of elastase have met with limited success. Hamsters injected intravenously with nonfatal doses of pancreatic elastase fail to show histologic changes characteristic of emphysema (Schuyler et al. 1978) and do not manifest detectable reductions in lung elastin (Ip et al. 1980). However, elastic recoil is lost at low lung volumes (Schuyler et al. 1978). Fierer et al. (1976) has noted enlargements in the airspaces of rats treated intravenously with large doses (330 U) of pancreatic elastase. They also found increases in the mean linear intercepts and rarefaction of the amorphous components of elastin within the lungs. It is doubtful, however, if proportionally similar intravenous levels of pancreatic elastase occur in humans with pulmonary emphysema.

Polymorphonuclear Leukocyte Elastase

Polymorphonuclear leukocytes (PMN) appear to be a more plausible source of endogenous elastase in the human lung than the pancreas, and are more likely to be incriminated in the pathogenesis of naturally occurring pulmonary emphysema. PMNs contain elasto-

lytic enzymes (Janoff 1973; Ohlsson and Ohlsson 1974; Rindler-Ludwig et al. 1974) that can be released in active form within the lung. Experimental studies have clearly demonstrated the ability of PMN elastase to degrade lung elastin and to induce emphysematous lesions in animals.

Marco et al. (1971) and Mass et al. (1972) induced experimental emphysema in dogs by the administration of aerosolized crude leukocyte homogenates. Using purified human leukocyte elastase, Janoff et al. (1977) demonstrated the ability of the enzyme to digest dog lung elastin *in vitro* and to cause significant dilation of terminal respiratory structures when instilled into isolated perfused dog lungs. The *in vivo* intratracheal instillation of human leukocyte elastase in dogs produces foci of alveolar destruction within 90 minutes of administration (Janoff et al. 1977). Senior et al. (1977) studied the effects of intratracheally injected human leukocyte elastase on hamsters and found a reduction in lung elastin in treated animals, as well as mild patchy airspace dilation. Sloan et al. (1981) were able to show that purified dog leukocyte elastase could also produce emphysematous lesions in dogs when instilled endobronchially.

Guenter et al. (1981) developed a dog model of experimentally induced emphysema that avoided the necessity of intratracheal instillation of enzymes. They repetitively injected *E. coli* endotoxin intravenously, thereby inducing extensive leukocyte sequestration within the lungs of the dogs. A previous study had shown that the sequestered cells degranulate and disintegrate within the vascular bed (Coalson et al. 1970). Histologic studies of these dogs revealed mild airspace destruction and prominent intra-alveolar fenestrations.

Alveolar Macrophage Elastase

In a widely cited article (Mass et al. 1972), dog alveolar macrophage homogenates (obtained by the method of Brain 1970), administered to two mongrel dogs produced "some dilatation and nonuniformity in the size of the airspaces accompanied by some alveolar wall destruction" in one of the dogs. The other dog showed no evidence of emphysema.

In spite of the paucity of animal data, the pulmonary alveolar macrophage (PAM) has been the focus of much investigation. Both experimental and clinical evidence is available that implicates this cell in the pathogenesis of pulmonary emphysema.

Two possible mechanisms by which macrophages may mediate tissue injury are being actively studied. One mechanism involves the release of elastolytic enzymes followed by unrestrained proteolysis. The second mechanism involves either a direct or an indirect injury

following the release of toxic forms of partially reduced oxygen such as superoxide anions, hydroxyl radicals, and hydrogen peroxide.

The ability of human alveolar macrophages to synthesize and secrete an elastolytic enzyme distinct from PMN elastase is the subject of controversy. Although human alveolar macrophages have been shown to synthesize a metalloprotease distinct from the serine protease (elastase) of the PMNs (DeCremoux et al. 1978), its hydrolytic activity against insoluble elastin substrate has not been conclusively demonstrated (Hinman et al. 1980; Levine et al. 1976). Interpretation of the observation that human alveolar macrophages raised in cell culture systems secrete an enzyme with true elastolytic activity against insoluble elastin (Rodriguez et al. 1977; DeCremoux et al. 1978) is complicated by the fact that alveolar macrophages bind and internalize PMN elastase (Campbell and Greco 1982; White et al. 1982; Campbell and Wald 1983). Hinman et al. (1980) detected a calcium-dependent metalloprotease in the culture medium and in the cell lysates of human alveolar macrophages and initially demonstrated elastolytic activity against synthetic elastin substrate and soluble elastin by both the culture medium fluid and the cell lysates. However, after 3 and 5 days of culture, no detectable activity against insoluble elastin was evident. The authors calculated that the initial elastolytic activity observed could be quantitatively explained by PMN contamination. The recognition that human alveolar macrophages internalize human PMN elastase (Campbell and Greco 1982; White et al. 1982; Campbell et al. 1979; Campbell and Wald 1983) and that the internalized PMN elastase retains enzymatic activity for at least 48 hours (McGowan et al. 1983) suggests an alternative explanation.

Green et al. (1979) subcultured human alveolar macrophages for 3 months and found measurable elastase activity against solubilized elastin during the entire period. They concluded that the elastase activity appeared to be synthesized continuously rather than being internalized from external sources.

In summary, human alveolar macrophages release elastolytic enzymes capable of digesting connective tissue. Whether the elastase released by these cells represents an enzyme synthesized *de novo* or a previously internalized PMN elastase is uncertain and requires further study.

Human alveolar macrophages, especially from cigarette smokers, secrete highly reactive oxygen species (Hoidal et al. 1979a) that are capable of directly injuring endothelial cells (Sacks et al. 1978) and fibroblasts (Hoidal et al. 1981) and of inactivating α_1 AT (Carp and Janoff 1979, Janoff 1979a).

Whole cigarette smoke inhibits PMN chemotaxis *in vitro* in a dose-dependent manner (Bridges et al. 1977). However, when alveolar macrophages are exposed to cigarette smoke either *in vitro* or *in*

vivo, they release a PMN chemotactic factor (Hunninghake et al. 1980c) (see above).

Protease-Antiprotease Hypothesis

The protease-antiprotease hypothesis proposes that enzymatic digestion of lung parenchyma occurs as a direct consequence of a genetic or acquired imbalance of the protease-antiprotease system and that the subsequent repair of connective tissue is unable to return the structures to normal. This hypothesis derives principally from two observations: (1) people genetically deficient in α_1 AT (Laurell and Eriksson 1963), the major antielastase of the lower respiratory tract of humans (Gadek et al. 1981a), are at greatly increased risk of developing pulmonary emphysema, and (2) proteolytic enzymes produce physiologic and anatomic lesions resembling emphysema when administered to experimental animals (Gross et al. 1965). Attempts to integrate the clearly established relationship of cigarette smoking and pulmonary emphysema with the protease-antiprotease hypothesis have led investigators to search for ways in which smoking perturbs this balance.

Increased Elastase Owing to the Cellular Response to Smoke

At least five variables, aside from the genetically determined level of antiprotease activity, could influence the elastase burden of the lungs. These variables include (1) an increase in the number of elastase-containing cells within the lung, (2) an increase in the quantity of prepackaged or newly synthesized elastase per cell, (3) the quantity of elastase released from the cells, (4) the proximity of the elastase to suitable substrate, and (5) the extracellular milieu (i.e., pH, ionic strength, and factors such as platelet factor 4).

Number of Cells

As discussed earlier, the human cigarette smoker has increased numbers of alveolar macrophages in the bronchoalveolar lavages compared with nonsmokers (Rodriguez et al. 1977; Harris et al. 1975; Reynolds and Newball 1974; Hoidal and Niewoehner 1982). Holt and Keast (1973b) found sustained elevations of pulmonary macrophages in mice exposed to cigarette smoke. Cigarette smoke has been shown to recruit PMNs into the airways (Kilburn and McKenzie 1975; Rylander 1974) and to induce alveolar macrophages to release a chemotactic factor for PMNs (Hunninghake et al. 1980c). The circulating PMNs are reported to be increased in cigarette smokers (Corre et al. 1971; Galdston et al. 1977). Hunninghake et al. (1980c) and Reynolds and Newball (1974) found increased numbers of PMNs in the lavage fluid of smokers, but Hoidal and Niewoehner (1982) reported similar numbers of PMNs in the lavages of cigarette

smokers and nonsmokers. Hunninghake and Crystal (1983) obtained isolated cell suspensions from the bronchoalveolar lavage fluids and from open lung biopsies of nonsmokers and cigarette smokers with both normal lung parenchyma and sarcoidosis. They found a significantly increased number of neutrophils and macrophages in the lavage fluid and in the biopsy specimens from cigarette smokers as compared with nonsmokers, both in patients with normal lung parenchyma and in those with sarcoidosis.

Elastase Content

Harris et al. (1975) found an increase in the elastase-like esterase and protease activity of macrophages obtained from smokers as compared with nonsmokers. Galdston et al. (1977) found the PMN elastase levels of circulating PMNs to be elevated in patients with chronic obstructive lung disease and suggested that the intracellular elastase levels may be genetically determined (Galdston et al. 1973). Other investigators (Lam et al. 1979; Rodriquez et al. 1979) reported similar findings, but Kramps et al. (1980) failed to find any correlation between the PMN elastase levels and obstructive lung disease in PiZZ patients, although they did note a difference in PiMM patients. Lonky et al. (1980) demonstrated that dogs infected with Type 3 pneumococcus had increased PMN elastase-like esterase activity within their cells, suggesting an acute phase reaction.

Release

A variety of mechanisms may lead to the extracellular release of lysosomal contents. These include cell lysis, regurgitation during phagocytosis, reverse endocytosis, humoral mediation, and cytochalasin B treatment of cells (Klebanoff and Clark 1978). Wright and Gallin (1979) showed that migration of PMNs is associated with the leakage of various enzymes. Sandhaus (1983) found that migrating human neutrophils degrade elastin *in vitro* in the presence or absence of human α_1 AT. A similar mechanism may occur during neutrophil migration *in vivo*. Hutchison et al. (1980) found that the soluble fraction of cigarette smoke suppressed the release of lysosomal enzymes (acid phosphatase and acid ribonuclease) from PMNs obtained from healthy persons, but not from the PMNs of emphysematous patients. Blue and Janoff (1978) demonstrated that the water-insoluble fraction of cigarette smoke has a cytotoxic effect on PMNs *in vitro* and causes them to release their lysosomal contents, including beta-glucuronidase, acid phosphatase, and elastase. Eliraz et al. (1977) found that canine alveolar macrophages and PMNs, when stimulated with the water-soluble fraction of cigarette smoke, secrete elastase. Abboud et al. (1983), however, compared the release of elastase and β -glucoaminodase from PMNs obtained from ciga-

rette smokers and with that from nonsmokers and found no differences. In vitro stimulation of these cells by either phagocytosis or chemotactic polypeptides did not alter the results. These researchers concluded that chronic smoking does not affect neutrophil elastase release in vitro and that among smokers there is no significant relationship between in vitro neutrophil elastase release and abnormalities in lung function. They speculated that some of the differences between studies may be related to experimental conditions, such as the concentrations of cigarette smoke.

Because the mechanisms involved in the release of intracellular contents are complex and the representativeness of in vitro conditions to in vivo events is uncertain, definite conclusions await further studies.

Proximity

Elastolytic activity is conditioned by the absorption of elastase onto elastin substrate (Robert and Robert 1970); the adsorption, in turn, results from the electrostatic attraction between negatively charged carboxylate groups of elastin and positively charged groups of elastase (Hall and Czerkowski 1961; Gertler 1971). Campbell et al. (1982) found that α_1 AT has less inhibitory activity against PMN elastase derived from cells in contact with substrate than against PMN elastase free in solution. They reasoned that the partial exclusion of protease inhibitors from the PMN-connective tissue interface may account for this phenomena and may be an important factor in elastase-mediated injury. Focusing more on the macroenvironment within the lung, Janoff et al. (1983c) found that the bronchoalveolar lavage fluids of young asymptomatic cigarette smokers contain significantly more elastase activity than the lavage fluids from nonsmokers. Kucich et al. (1983) found that the serum lung elastin-derived peptides were elevated in some smokers and most patients with COLD, suggesting that elastolysis may be taking place in smokers and COLD patients.

Milieu

A number of in vitro experiments have examined the chemical and physical conditions that modify neutrophil elastase kinetics. Lestienne and Bieth (1980) demonstrated that human leukocyte elastase activity is activated in the presence of substrate excess, hydrophobic solvents, and increasing ionic strength. The adsorption of sodium dodecyl sulfate (SDS), a hydrophobic, anionic ligand, onto the surface of elastin enhances the elastolytic activity of pancreatic elastase (Kagan et al. 1972). Lonky et al. (1978) showed that platelet factor 4 (PF₄) in physiologic concentrations is capable of in vitro stimulation of human neutrophil elastase (HLE) against lung elastin. Low doses

of HLE instilled intratracheally in hamsters failed to induce physiologic, morphologic, or biochemical changes, but following the addition of PF₄, a significant injury was evident, and the elastin content of the lung was lowered by 20 percent (Lonky et al. 1978). Boudier et al. (1981) demonstrated that human leukocyte cathepsin-G, an enzyme in the azurophilic granules that possesses little intrinsic elastolytic activity, stimulates the rate of solubilization of human lung elastin by HLE. The elastolytic activity increased by more than five times the HLE rate when the HLE-cathepsin-G mixture was present in equimolar concentrations. The relevance of these findings to the physiologic conditions that prevail in vivo requires further study.

Laurent et al. (1983) recently discovered that the water-soluble components of filtered cigarette smoke suppress, in a dose-dependent manner, the lysyl oxidase-catalyzed oxidation of the epsilon-amino groups of lysine residues in tropoelastin. This step is essential for the formation of covalent cross-links between neighboring elastin polypeptide chains that, in turn, are necessary for normal elastic strength within the lung.

Decreased Antiprotease Owing to Oxidation

A comprehensive review of the role of oxidative processes in emphysema has recently been published by Janoff et al. (1983a). In vitro studies have revealed that oxidants such as chloramine T (Abrams et al. 1981) or ozone (Johnson 1980) cause a loss in the inhibitory capacity of α_1 AT for neutrophil elastase. The mechanism of inactivation has been identified as the oxidation of methionine and tyrosine residues (Johnson and Travis 1979; Cohen 1979; Carp and Janoff 1978) within the α_1 AT molecule. Chloramine T, when administered differentially to dogs, reduces EIC > TIC of serum and also results in emphysema (Abrams et al. 1981). Cigarette smoke is also known to contain oxidants (Stedman 1968; Pryor et al. 1983). Aqueous solutions of cigarette smoke reduce the elastase inhibitory capacity of human serum (Carp and Janoff 1978) and result in less binding of elastase to α_1 AT in vitro (Carp and Janoff 1978). Some investigators have found that the α_1 AT activity is reduced in the lavage fluids (BALF) obtained from human cigarette smokers and from rats exposed to cigarette smoke (Gadek et al. 1979; Carp et al. 1982; Janoff et al. 1979a). Stone et al. (1983) reported similar levels of functional α_1 -antitrypsin in the bronchoalveolar lavage fluids of human smokers and nonsmokers. Janoff and Chan (1984) have suggested that this difference in results may reflect the timing of the lavage in these studies, as rats chronically exposed to cigarette smoke had rapid inactivation of α_1 -antitrypsin following smoke exposure, but also had a more rapid recovery of α_1 -antitrypsin activity than did rats acutely exposed to smoke. Stone et al. also

recognized that their study may not have detected a reduction in α_1 -antitrypsin activity if it was accompanied by a rapid recovery to normal levels. Methionine sulfoxide peptidase, an enzyme present in human PMNs, can reactivate α_1 AT oxidized by chloramine T or by the myeloperoxidase system, but α_1 AT exposed to cigarette smoke plus peroxide (Carp et al. 1983) has been shown to be either resistant to reactivation by the myeloperoxidase system (Carp et al. 1983) or incompletely reactivated (James et al. 1984).

Human ceruloplasmin has been shown to prevent myeloperoxidase mediated oxidation of α_1 AT under specified conditions of pH and solvency (Taylor and Oey 1982), although the role played by ceruloplasmin in limiting oxidation by phagocytes *in vivo* is unclear. Taylor et al. (1983) examined plasma and leukocyte lysosomal samples from a group of COLD patients and measured the ability of these samples to inhibit lipid peroxidation. While they reasoned that inhibitors of peroxidation could protect α_1 AT from inactivation by neutralizing lipid free radicals, they found that inhibition required factors from both plasma and lysosomal extracts and that the factor was not ceruloplasmin. Two of ten emphysematous patients had reduced plasma factor activity, and one of these patients also had reduced lysosomal factor. Controls had normal values for both of these factors.

Galdston et al. (1984) examined serum ceruloplasmin concentrations and antioxidant activity in male and female smokers. Smokers of both sexes had higher serum ceruloplasmin concentrations than did nonsmokers; women in both smoking categories had higher concentrations than their male counterparts. Serum antioxidant activity showed a significant positive correlation with serum ceruloplasmin levels; however, for comparable ceruloplasmin concentrations, serum antioxidant activity was significantly lower in smokers than in nonsmokers of both sexes. The researchers suggest that cigarette smoking may cause partial inactivation of serum antioxidant activity that is accompanied by an insufficient increase in ceruloplasmin concentration.

Endogenous phagocytes are also capable of generating oxidants (Babior 1978; Klebanoff and Clark 1978). The phagocytic enzyme myeloperoxidase, in the presence of hydrogen peroxide and halide ions, oxidatively inactivates α_1 AT (Matheson et al. 1979, 1981). Smoking, as described above, elevates the oxidative metabolism in lung macrophages (Hoidal and Niewoehner 1982; Fox et al. 1979; 1980; 1981).

Thus, it is clear that the oxidants present in cigarette smoke and the lung macrophages of smokers can inactivate α_1 AT. This inactivation, coupled with the increased elastase burden that may result from the inflammatory cell response of the lung to smoke, could tip

the balance of the protease–antiprotease system in the direction of elastin degradation.

Explanation for Upper Lobe Distribution

In accord with the protease–antiprotease hypothesis, emphysematous lesions result from the unrestrained proteolytic digestion of connective tissue elements. The regional distribution of lesions within the lung is thought to be conditioned by both biochemical and physiological variables.

The predilection of lower lobe involvement in persons with α_1 AT deficiency is hypothesized to result from an increased number of elastase-containing cells because of the higher vascular perfusion to this area in erect man. This excess could occur because of the deposition of senescent leukocytes in these areas of higher blood flow (Guenter et al. 1981). In addition, inhaled particulates preferentially deposit in the lower lobes (Milic-Emili et al. 1966; Dollfuss et al. 1967), and the leukocytes release their enzyme extracellularly during the ingestion of these particulates. Because of the genetic deficiency of α_1 AT, the proteolytic activity is unopposed and destruction occurs.

The predominance of upper lobe lesions in cigarette smokers with normal systemic levels of α_1 AT is again thought to result from variations of ventilation and perfusion within the lung (Cockcroft and Horne 1982). However, in normal individuals the proteolytic activity due to the excess particulate deposition in the bases is inhibited by α_1 AT that is replenished by the increased vascular perfusion also occurring in the bases. The upper lobes, although less well ventilated than the lower lobes, nevertheless have higher ventilation:perfusion ratios because of the proportionately greater fall in perfusion. The oxidative inactivation of α_1 AT by cigarette smoke in the upper lobes therefore may not be compensated by vascular repletion of the inactivated α_1 AT, and an imbalance of protease–antiprotease may occur. The upper lobe injury may then be magnified by mechanical stresses caused secondary to the negative intrapleural pressures generated by gravitational forces in erect man (West 1971).

Animal Models of Emphysema

Spontaneous Emphysema

Emphysema occurs spontaneously in animals in forms resembling the types seen in human disease (Karlinsky and Snider 1978). However, the low incidence and unpredictable occurrence of disease in animals greatly limit their utility as experimental models.